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The stability of mesna in beverages and syrup for oral administration*

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Summary. We evaluated the stability of the aqueous formulation of mesna during storage in syringes and after dilution in beverages and syrups. Measurements of the concentrations of mesna showed that the undiluted formulation was stable for at least 9 days in standard polypropylene syringes at 5°, 24°, and 35° C. There was no detectable oxidation of mesna to dimesna over the course of at least 1 week when mesna was diluted 1:2 and 1:5 in syrups and incubated at 24° C in capped tubes. Concentration changes were clinically negligible for 1:2, 1:10, and 1:100 dilutions of mesna in six carbonated drinks, two juices, and milk after incubation for 24 h at 5° C. Thus, the aqueous mesna formulation is stable when diluted and stored in a variety of beverages and syrups under conditions suitable for oral administration.

Introduction

Mesna is effective in preventing ifosfamide-associated hemorrhagic cystitis [11]. Standard practice is to give mesna intravenously immediately before the injection of ifosfamide and then at 4-h intervals. To avoid hospitalization solely for the injection of mesna, oral use of the drug has been advocated [1, 3]. In a clinical study of 45 lung cancer patients given oral mesna, Araujo and Tessler [1] reported only 10 episodes of asymptomatic microscopic hematuria and no gross hematuria during 88 courses of ifosfamide. Bioavailability studies also support the potential substitution of oral for intravenous mesna. Burkert et al. [3] found that more than one-third of multiple oral doses of mesna given to 18 volunteers and 5 patients was excreted in the active thiol form. About 24% of a 400-mg oral mesna dose was found by Jones et al. [6] to be excreted within 4 h. In a study of six volunteers given 800 mg of mesna by both the oral and the intravenous route [5], the excretion of mesna after oral dosing ranged from 29% to 81% of that produced by intravenous injection.

Mesna can be diluted in a beverage or syrup to mask its sulfurous taste. However, dilution of the aqueous formulation also reduces the concentration of the accompanying sulfhydryl stabilizing agent ethylenediaminetetraacetic acid (EDTA), increasing the possibility of oxidation of mesna to the disulfide form, dimesna, and of possible reactions between mesna and other constituents in the beverages. We therefore evaluated the stability of the aqueous formulation of mesna during storage in syringes and after dilution in beverages and syrups.

Materials and methods

Materials. Mesna (Mesnex, Asta Pharma AG, Frankfurt) was supplied in 10-ml ampuls by Bristol-Myers Oncology Division, Indianapolis, as a solution containing 100 mg drug/ml and 0.25 mg EDTA/ml. Dimesna was a gift of Asta Pharma AG. Disodium 2-nitro-5-thiosulfobenzoate was prepared from 5,5′-dithiobis(2-nitrobenzoic acid) (Sigma Chemical Company, St. Louis) as described by Thannhauser et al. [13].

Analytical determinations. Mesna and dimesna were measured by colorimetric procedures for the determination of thiols [9] and of total thiols and disulfides [13], which were adapted to an automated clinical analyzer (American Monitor Diagnostic Corporation, Indianapolis) [4]. In these two procedures, sample volumes of 50 µl were incubated with reagent volumes of 0.6 ml at 37° C for 45 s for Ellman's reagent and for 11.6 min for disodium 2-nitro-5-thiosulfobenzoate, respectively. These procedures were calibrated before each set of analyses by making a 1:1,000 dilution from a fresh vial containing 100 mg Mesnex/ml to obtain a 0.1-mg/ml (0.609 mM) solution. Test samples were diluted to approximate this standard concentration and were analyzed in triplicate to obtain an average value.

Definition of stability. We monitored the stability of mesna for at least 1 week in syringes and syrups and for 24 h in beverages, or until the occurrence of at least a 10% decrease in mesna concentration accom-

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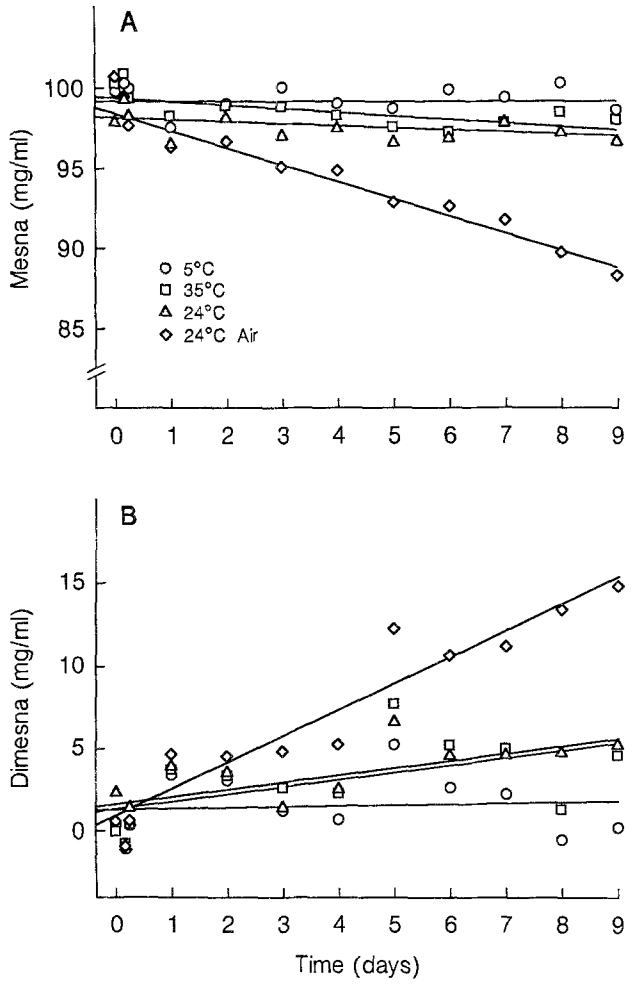

Fig. 1 A, B. Stability of mesna in syringes. **A** Concentration-time slopes determined by linear regression differ significantly from zero for mesna stored at 24° C with exposure to air ($P < 0.0001$; 2-sided t-test) and at 35° C without exposure to air ($P = 0.006$). **B** Differences were also significant for dimesna under the same conditions ($P < 0.0001$ and $P = 0.03$, respectively). Values represent the average of triplicate measurements from duplicate syringes containing the undiluted mesna formulation.

panied by the appearance of a corresponding amount of dimesna. For accurate detection of the target values associated with a 10% change, two aqueous control samples (a solution containing 0.1 mg mesna/ml and a solution containing 0.09 mg mesna/ml aand 0.01 mg dimesna/ml were prepared daily.

Stability studies in syringes. Mesnex (10 ml) was transferred from sealed ampules to 20-ml polypropylene syringes (Becton Dickenson, Rutherford, N. J.) and incubated at 5°, 24°, and 35° C in duplicate. The effect of adding an equal volume of air to the mesna was tested in an additional set of duplicate syringes at room temperature. Mesna and dimesna concentrations were measured in 0.5-ml aliquots that were removed from each syringe at 0, 4, 6, and 24 h and then daily for 9 days. As a positive control for the degradation of mesna under the conditions of the study, an aqueous solution containing 0.1 mg mesna/ml was prepared gravimetrically without EDTA and the concentration of mesna was determined at 0, 0.5, 4, 6, 9, 24, 48, 72, and 96 h.

Stability studies in syrups and beverages. Dilutions of Mesnex in syrups and beverages were based on estimates of the lowest and highest reasonable clinical doses in volumes of beverage that could be easily consumed: a 0.1-g dose (equivalent to 20% of a corresponding dose of 1 g/m^2 ifosfamide in a child with a body surface area (BSA) of 0.5 m^2)

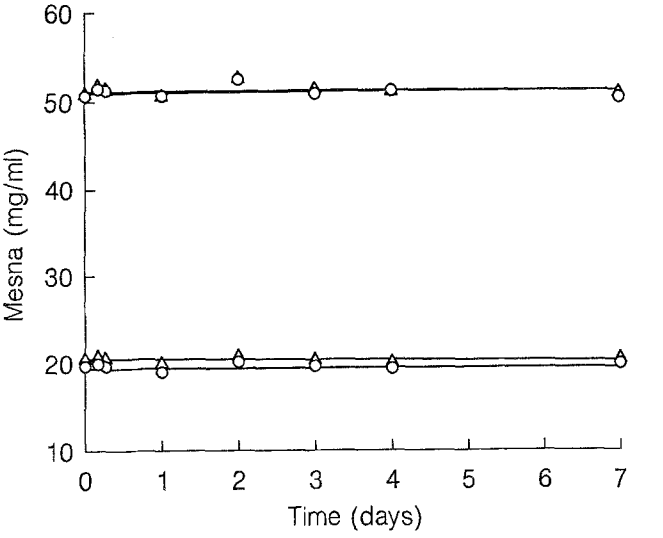

Fig. 2. Stability of mesna in syrup. Values represent the average of triplicate measurements from duplicate tubes containing the mesna formulation diluted 1:2 and 1:5 (v/v) in orange (○) and grape (△) syrup. The slopes determined by linear regression do not differ significantly from zero

and a 10-g dose (equivalent to 100% of a dose of 5 g/m^2 ifosfamide in an adult with a BSA of 2 m^2). The stability of 1:5 and 1:2 dilutions of mesna in grape- and orange-flavored syrups was tested over 1 week in capped glass tubes stored at 24° C. The stability of dilutions of mesna to 50, 10, and 1 mg/ml in three dark carbonated drinks (Coca Cola, Dr. Pepper, and Pepsi Cola), three clear carbonated drinks (7-Up, Sprite, and Schweppes ginger ale), two juices (orange and apple), and whole milk (chocolate and plain) was evaluated by adding 100, 10, and 1 ml Mesnex to 100, 90, and 99 ml, respectively, of beverage. Beverages were analyzed for mesna and dimesna concentrations both immediately and after incubation for 24 h in capped glass tubes stored at 5° C.

Results

Day-to-day variability in the analytical procedures was within the range required to detect a 10% decrease in mesna concentrations. The between-run coefficients of variation over 18 days for the 0.1- and 0.09-mg/ml control solutions of mesna were 1.43% and 1.61%, respectively. The between-run coefficient of variation for the 0.01-mg/ml dimesna control was higher (14.75%), partly because the dimesna concentrations were derived from the difference in values obtained from determinations of the concentration of thiols and the sum of thiols and disulfides; therefore, the analytical variability associated with the determination of dimesna included the sum of the variability for both assays. The concentration of the dimesna control was also >3 times greater than the limit of sensitivity for the method, judged as being 2 SD above the average background level for aqueous controls containing mesna but no dimesna. The within-day coefficients of variation for the triplicate measurements as determined by analysis of variance were 0.40%, 0.68%, and 0.76% for the 0.1 and 0.09-mg/ml mesna controls and the 0.01-mg/ml dimesna control, respectively.

The undiluted 100-mg/ml mesna formulation was stable in syringes at all temperatures tested, but mesna concentra-

Table 1. Changes in mesna and dimesna concentrations at 24 h after the addition of mesna to beverages

Beverage	Mesna concentration in beverages					
	1 mg/ml		10 mg/ml		50 mg/ml	
	Mesna	Dimesna	Mesna	Dimesna	Mesna	Dimesna
Dark carbonated drinks:						
Coca Cola	–4.4%	1.9%	–2.9%	0.4%	–2.9%	1.5%
Dr. Pepper	–0.9%	1.6%	0.8%	0.2%	–2.3%	2.2%
Pepsi Cola	0.4%	1.2%	–1.9%	1.0%	–0.9%	2.0%
Clear carbonated drinks:						
Sprite	–2.2%	1.1%	–3.0%	0.9%	–0.6%	0.9%
7-Up	0.7%	0.9%	0.3%	0.7%	0.4%	0.2%
Ginger ale	–1.7%	1.6%	–1.8%	0.4%	–0.6%	0.4%
Juices:						
Apple juice	–3.1%	4.4%	–1.6%	0.4%	0.1%	1.2%
Orange juice	–2.8%	1.0%	–5.2%	1.2%	–0.6%	0.3%
Milk:						
Whole milk	–8.2%	14.0%	–5.0%	1.4%	–3.5%	3.0%
Chocolate milk	–8.9%	6.2%	–4.3%	5.4%	1.8%	–0.3%

Values for mesna represent the average percentage of change in mesna concentration for two samples measured in triplicate at 0 and 24 h. Values for dimesna represent twice the dimesna concentration at 24 h (to obtain the number of mesna equivalents), expressed as a percentage of the mesna concentration in the beverage

tions decreased by 10% within 8 days when air was added to syringes stored at 24° C (Fig. 1A); corresponding increases in dimesna concentrations were also observed (Fig. 1B). Although the slopes determined by linear regression for syringes stored at 35° C also differed significantly from zero, the maximal change in mesna and dimesna concentrations was <4%. By contrast, in the 0.1-mg/ml solution of mesna prepared without EDTA and stored at 5° C in a capped glass tube, mesna concentrations decreased at a linear rate by 18%/day.

There was no detectable change in the concentration of the mesna formulations diluted 1:5 and 1:2 in syrup and stored at 24° C in capped tubes for at least 1 week (Fig. 2). When mesna was added to beverages (Table 1), drug concentration changes after 24 h were negligible, with one exception. When mesna was diluted 100-fold to 1 mg/ml in whole milk and chocolate milk, drug concentrations decreased after 24 h by 8.2% and 8.9%, respectively, accompanied by the appearance of dimesna in an amount equivalent to 14% and 6.2% of the mesna added to the beverage. Thus, about 10% of the mesna was oxidized to dimesna. Decrements in mesna concentration occurred in 23 of the 30 combinations of beverages and dilutions tested, whereas increments in dimesna occurred in 29 of 30 instances.

Analytical variability in the measurements presumably exceeded that for the aqueous controls for two reasons: increased background absorbance associated with beverage constituents such as the proteins and lipids in milk, and low dimesna concentrations that approached the limit of sensitivity of the assay. Differences in paired results reflected this variability. Despite variability in the data, decrements in mesna and corresponding increments in dimesna were found to be associated with increases in dilution using multivariate analysis of variance ($P = 0.026$, Wilk's lambda), indicating that mesna was less stable in more dilute solutions.

Discussion

The present results indicate that the aqueous mesna formulation is stable when diluted and stored in a variety of beverages and syrups under conditions suitable for oral administration. Even 100-fold dilutions of the pharmaceutical formulation in beverages showed little change over 24 h, with the exception of milk, in which the decrease in mesna approached 10%, suggesting that dilute solutions of mesna in milk should not be stored for more than 1 day. Factors that may have jointly contributed to the stability of mesna include an acidic pH, a protein-free matrix, carbon dioxide saturation, and EDTA, as suggested by the smaller mesna concentration changes after 24 h in juices and acidic carbonated beverages as compared with milk and by the loss of mesna in the control that did not contain EDTA. Minimizing the exposure of mesna to air during storage slowed its conversion to dimesna.

Evidence from animal and clinical studies suggests that an oral mesna formulation may remain therapeutically active despite the oxidation of mesna to dimesna. Dimesna is absorbed from the gastrointestinal tract and undergoes reduction to mesna during or after absorption [2, 7]. In a study of oral dimesna given to six volunteers, 18% of the dose was excreted as free thiol within 24 h [12]. Thus, even if a substantial proportion of mesna had been oxidized under the conditions studied, the resulting dimesna presumably would have retained some therapeutic activity.

The stability of mesna diluted in syrups indicates that patients can store mesna in syrup at room temperature and then either consume the syrup directly or add it to a bever-

age in the appropriate amount at designated times. Aliquots of mesna can also be provided to patients in separate polypropylene syringes for addition to beverages. These data also support the feasibility of delivering mesna with ifosfamide to ambulatory patients using infusion pumps as suggested by Rowland et al. [10] and Radford et al. [8], who reported that aqueous solutions of ifosfamide undergo negligible change for at least 1 week. Studies are under way in our laboratory to identify a dosage and frequency of oral mesna therapy that produces uroprotective concentrations equivalent to those obtained after intravenous administration.

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